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Application of Next-Generation Sequencing to Food Authenticity Testing - Study of Adulterated Beef Samples Using Ion GeneStudio S5 Food Protection System

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ABSTRACT

Following the UK/EU Horse-meat issues of 2013, where a significant amount of horse DNA was found in a number of processed beef products there has been an increased need for routine non-targeted species detection methods. In recent years Next-Generation Sequencing (NGS) has been promoted as a useful technique to identify species present in samples containing a mixture of species. Very few studies have looked into application of processed meat products where DNA can be highly degraded. This study applies a commercial NGS system to a range of spiked meat product samples processed to industry standard conditions. The samples consisted of lean beef spiked with varying levels of pork and horse muscle was used to prepare raw, burger, canned meat and cottage pie sample types. Multiple DNA extracts were prepared from each sample type and NGS was performed using SGS™ All Species Meat Analysis kit in conjunction with Ion Chef™ Food Protection Instrument and Ion GeneStudio™ S5 Food Protection System. Results will be presented and relevance to food screening will be discussed.

INTRODUCTION

Next Generation Sequencing (NGS) has been introduced in recent years as a very powerful DNA-based method for species identification in food products. However, the use of NGS as a food protection tool requires the development of optimized, fit for purpose, workflow to ensure reliability of results and to maximize the advantages of this high throughput method. To take advantage of the non-targeted and massive sequencing output obtained by NGS a food protection specific end to end workflow has been developed to enable identification of meat, fish and plant species in food products.

In the present study the Thermo Scientific™ NGS Food Authenticity Workflow (Figure 1) for meat species identification is used for the detection of adulterated beef samples. An application which has particular relevance given the EU horse-meat issues of 2013 in which food advertised at containing beef where found to contain significant amounts of horse meat and to a lesser degree other undeclared meat such as pork. The resulting scandal highlighted a significant breakdown in the traceability of the food supply food chain and called into question brand integrity within the food industry.

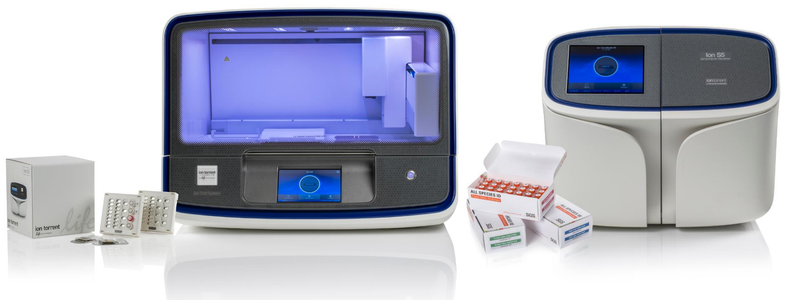

Figure 1. Ion Chef™ Food Protection Instrument, Ion GeneStudio™ Food Protection NGS System and SGS™ All Species ID Analyser Kits

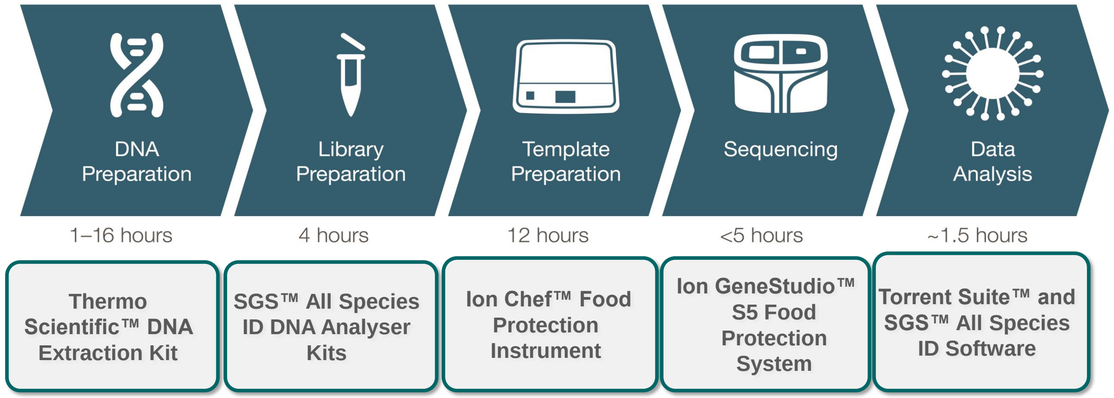

Figure 2. Thermo Scientific NGS Food Authenticity Workflow overview

MATERIALS AND METHODS

A number of samples of beef with different amounts of horse and pork meat were prepared and then subjected to different processing levels.

Samples were homogenized and a DNA extraction step performed on 200 mg sample. Libraries for sequencing were prepared using SGS™ All Species Meat Analyser kit (Thermo Fisher Scientific). Unique barcodes (i.e. molecular tags) were added to each sample to enable sequencing and analysis of several samples within the same sequencing run.

A fully automated templating reaction on the Ion Chef Food Protection instrument (Thermo Fisher Scientific) was performed to prepare the sample libraries for sequencing on the Ion Chips. Sequencing was performed on the Ion GeneStudio S5 Food Protection System (Thermo Fisher Scientific) relying on semi-conductor technology. Sequencing results were mapped against a database of species DNA of meat, fish and plant for data analysis. A comprehensive list of all species detected in a sample was generated by the SGS™ All Species ID software (Thermo Fisher Scientific).

RESULTS

The output from the Thermo Scientific NGS Food Authenticity Workflow was collated to show whether Horse or Pork meat was detected in the samples at the given contamination levels, as shown in Tables 1 & 2.

CONCLUSIONS

Results suggest that this NGS Food Authenticity Workflow is capable of detecting contamination down to 1% in processed samples although there may be some slight differences in sensitivities between species.
The results suggest that this system will not detect contamination at a level of 0.1% so may not be suitable in situations where there is a requirement for low level detection i.e. halal foods where levels down to 0.1% are often tested. Real-time PCR is used in targeted species detection and can detect down to 0.1%.

The method is suitable for screening and as an estimate of potential levels and should not be considered quantitative.

TRADEMARKS

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Horse %	Unprocessed (n=4)	Canned (n=4)	Burger (n=3)	Cottage Pie (n=3)	% Detection
10	4	4	3	3	**100**
2	4	4	3	3	**100**
1	2	1	0	2	**36**
0.1	0	0	0	0	**0**

Table 1. Percentage of horse contamination in mixed beef and horse samples

Pork %	Unprocessed (n=4)	Canned (n=4)	Burger (n=3)	Cottage Pie (n=3)	% Detection
10	4	4	3	3	**100**
2	4	4	3	3	**100**
1	4	3	3	3	**93**
0.1	0	0	0	0	**0**

Table 2. Percentage of pork contamination in mixed beef and pork samples

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